FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **221045US0PCT**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): 10/088502

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/06874	**2 October 2000**	**4 October 1999 (earliest)**

TITLE OF INVENTION
NEW USE

APPLICANT(S) FOR DO/EO/US
MORIGUCHI Akira et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PCT/IB/304
PCT/IB/308
Form PTO-1449
Request for Priority

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/088502	PCT/JP00/06874	221045US0PCT

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :			
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1040.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	15 - 20 =	0	x $18.00	**$0.00**	
Independent claims	11 - 3 =	8	x $84.00	**$672.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,562.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,562.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE =				**$1,562.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$1,562.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,562.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Surinder Sachar
Registration No. 34,423

22850

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

March 28 2002
DATE

DESCRIPTION

NEW USE

Technical Field

This invention relates to a new use of a plasminogen activator, which is useful in a medical field.

Background art

Various plasminogen activators are well known. And, a certain macrolide compound, i.e., tacrolimus, and its related compounds are known to have preventing or treating activity of cerebral infarction (USP 5,648,351).

Disclosure of Invention

This invention relates to a new use of plasminogen activators, for increasing an effect caused by interleukin 2 inhibitor (hereinafter, referred to IL-2 inhibitor).

Therefore, one object of the present invention is to provide a new use of a plasminogen activator for increasing an effect caused by IL-2 inhibitor.

Another object of this invention is to provide a method for increasing an effect caused by IL-2 inhibitor by administering an effective amount of a plasminogen activator.

A further object of this invention is to provide a use of a plasminogen activator for manufacturing a medicament for increasing an effect caused by IL-2 inhibitor.

Still further object of this invention is to provide a composition comprising a plasminogen activator, for increasing an effect caused by IL-2 inhibitor.

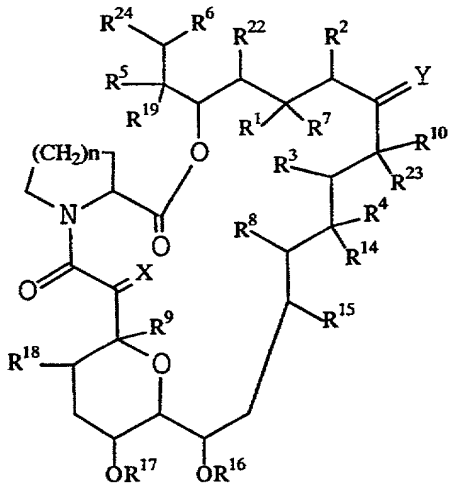
In the present invention, the "plasminogen activator" should not be limited and be considered to mean any compounds which can convert inactivate plasminogen to the protease plasmin. For example, tissue-type plasminogen activator(tPA), urokinase (UK), pro-urokinase, streptokinase, acylated streptokinase/tPA conjugates, etc.

The "IL-2 inhibitor" used in the present invention should not be limited and be considered to mean any ones possessing IL-2 inhibitory activity. The particular example is the one possessing an inhibitory activity on the production of IL-2. And the other is the one that inhibits the transmission of IL-2 signal.

The preferable "effect caused by IL-2 inhibitor" is a neuroptotective activity. Particularly, "the effect caused by IL-2 inhibitor" may be the treatment and prevention of acute or chronic cerebral neurodegenerative diseases, such as cerebral ischemic diseases and/or brain damage caused by ischemia.

Preferable "IL-2 inhibitor" is, for example, the tricyclic macrolide shown in EP-0184162, WO89/05303, WO93/05058, WO96/31514, and so on, the disclosure of which is incorporated herein by reference. It is well known that those tricyclic macrolides have strong IL-2 inhibitory activity.

As a particular example of the tricyclic macrolides compounds, the tricyclic compound of the following formula (I) can be exemplified.



(I)

(wherein each of adjacent pairs of R^1 and R^2, R^3 and R^4 , and R^5 and R^6 independently

(a) is two adjacent hydrogen atoms, but R^2 may also be an alkyl group or

(b) may form another bond formed between the carbon atoms to which they are attached;

R^7 is a hydrogen atom, a hydroxy group, a protected hydroxy group, or an alkoxy group, or an oxo group together with R^1;

R^8 and R^9 are independently a hydrogen atom or a hydroxy group;

R^{10} is a hydrogen atom, an alkyl group, an alkyl group substituted by one or more hydroxy groups, an alkenyl group, an alkenyl group substituted by one or more hydroxy groups, or an alkyl group substituted by an oxo group;

X is an oxo group, (a hydrogen atom and a hydroxy group), (a hydrogen atom and a hydrogen atom), or a group represented by the formula $-CH_2O-$;

Y is an oxo group, (a hydrogen atom and a hydroxy group), (a hydrogen atom and a hydrogen atom), or a group represented by the formula $N-NR^{11}R^{12}$ or $N-OR^{13}$;

R^{11} and R^{12} are independently a hydrogen atom, an alkyl group, an aryl group or a tosyl group;

R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, R^{22} and R^{23} are independently a hydrogen atom or an alkyl group;

R^{24} is an optionally substituted ring system which may contain one or more heteroatoms;

n is an integer of 1 or 2; and

in addition to the above definitions, Y, R^{10} and R^{23}, together with the carbon atoms to which they are attached, may represent a saturated or unsaturated 5- or 6-membered nitrogen, sulfur and/or oxygen containing heterocyclic ring optionally substituted by one or more groups selected from the group consisting of an alkyl, a hydroxy, an alkoxy, a benzyl, a group of the formula $-CH_2Se(C_6H_5)$, and an alkyl substituted by one or more hydroxy groups.

The definitions used in the above general formula (I) and the specific and preferred examples thereof are now explained and set forth in detail.

The term "lower" means, unless otherwise indicated, a group having 1 to 6 carbon atoms.

Preferable examples of the "alkyl groups" and an alkyl moiety of the "alkoxy group" include a straight or branched chain aliphatic hydrocarbon residue, for example, a lower alkyl group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, neopentyl and hexyl.

Preferable examples of the "alkenyl groups" include a

straight or branched chain aliphatic hydrocarbon residue having one double-bond, for example, a lower alkenyl group such as vinyl, propenyl (e.g., allyl group), butenyl, methylpropenyl, pentenyl and hexenyl.

Preferable examples of the "aryl groups" include phenyl, tolyl, xylyl, cumenyl, mesityl and naphthyl.

Preferable protective groups in the "protected hydroxy groups" and the "protected amino" are 1-(lower alkylthio)-(lower)alkyl group such as a lower alkylthiomethyl group (e.g., methylthiomethyl, ethylthiomethyl, propylthiomethyl, isopropylthiomethyl, butylthiomethyl, isobutylthiomethyl, hexylthiomethyl, etc.), more preferably C_1 -C_4 alkylthiomethyl group, most preferably methylthiomethyl group;

trisubstituted silyl group such as a tri(lower)alkylsilyl (e.g., trimethylsilyl, triethylsilyl, tributylsilyl, tert-butyldimethylsilyl, tri-tert-butylsilyl, etc.) or lower alkyl-diarylsilyl (e.g., methyldiphenylsilyl, ethyldiphenylsilyl, propyldiphenylsilyl, tert-butyldiphenylsilyl, etc.), more preferably tri(C_1-C_4)alkylsilyl group and C_1-C_4 alkyldiphenylsilyl group, most preferably tert-butyldimethylsilyl group and tert-butyldiphenylsilyl group; and an acyl group such as an aliphatic, aromatic acyl group or an aliphatic acyl group substituted by an aromatic group, which are derived from a carboxylic acid, sulfonic acid or carbamic acid.

Examples of the aliphatic acyl groups include a lower alkanoyl group optionally having one or more suitable substituents such as carboxy, e.g., formyl, acetyl, propionyl, butyryl, isobutyryl, valeryl, isovaleryl, pivaloyl, hexanoyl, carboxyacetyl, carboxypropionyl, carboxybutyryl,

carboxyhexanoyl, etc.;

a cyclo(lower)alkoxy(lower)alkanoyl group optionally having one or more suitable substituents such as lower alkyl, e.g., cyclopropyloxyacetyl, cyclobutyloxypropionyl, cycloheptyloxybutyryl, menthyloxyacetyl, menthyloxypropionyl, menthyloxybutyryl, menthyloxypentanoyl, menthyloxyhexanoyl, etc.; a camphorsulfonyl group; or a lower alkylcarbamoyl group having one or more suitable substituents such as carboxy or protected carboxy, for example, carboxy(lower)alkylcarbamoyl group (e.g., carboxymethylcarbamoyl, carboxyethylcarbamoyl, carboxypropylcarbamoyl, carboxybutylcarbamoyl, carboxypentylcarbamoyl, carboxyhexylcarbamoyl, etc.), tri-(lower)alkylsilyl(lower)alkoxycarbonyl(lower)alkylcarbamoyl group (e.g., trimethylsilylmethoxycarbonylethylcarbamoyl, trimethylsilylethoxycarbonylpropylcarbamoyl, triethylsilylethoxycarbonylpropylcarbamoyl, tert-butyldimethylsilylethoxycarbonylpropylcarbamoyl, trimethylsilylpropoxycarbonylbutylcarbamoyl, etc.) and so on.

Examples of the aromatic acyl groups include an aroyl group optionally having one or more suitable substituents such as nitro, e.g., benzoyl, toluoyl, xyloyl, naphthoyl, nitrobenzoyl, dinitrobenzoyl, nitronaphthoyl, etc.; and

an arenesulfonyl group optionally having one or more suitable substituents such as halogen, e.g., benzenesulfonyl, toluenesulfonyl, xylenesulfonyl, naphthalenesulfonyl, fluorobenzenesulfonyl, chlorobenzenesulfonyl, bromobenzenesulfonyl, iodobenzenesulfonyl, etc.

Examples of the aliphatic acyl groups substituted by an aromatic group include ar(lower)alkanoyl group optionally having

one or more suitable substituents such as lower alkoxy or trihalo(lower)alkyl, e.g., phenylacetyl, phenylpropionyl, phenylbutyryl, 2-trifluoromethyl-2-methoxy-2-phenylacetyl, 2-ethyl-2-trifluoromethyl-2-phenylacetyl, 2-trifluoromethyl-2-propoxy-2-phenylacetyl, etc.

More preferable acyl groups among the aforesaid acyl groups are C_1-C_4 alkanoyl group optionally having carboxy, cyclo(C_5-C_6)alkoxy(C_1-C_4)alkanoyl group having two (C_1-C_4) alkyls at the cycloalkyl moiety, camphorsulfonyl group, carboxy-(C_1-C_4)alkylcarbamoyl group, tri(C_1-C_4)alkylsilyl(C_1-C_4)alkoxycarbonyl(C_1-C_4)-alkylcarbamoyl group, benzoyl group optionally having one or two nitro groups, benzenesulfonyl group having halogen, or phenyl(C_1-C_4)alkanoyl group having C_1-C_4 alkoxy and trihalo(C_1-C_4)alkyl group. Among these, the most preferable ones are acetyl, carboxypropionyl, menthyloxyacetyl, camphorsulfonyl, benzoyl, nitrobenzoyl, dinitrobenzoyl, iodobenzenesulfonyl and 2-trifluoromethyl-2-methoxy-2-phenylacetyl.

Preferable examples of the "5- or 6-membered nitrogen, sulfur and/or oxygen containing heterocyclic ring" include a pyrrolyl group and a tetrahydrofuryl group.

R^{24} is an optionally substituted ring system which may contain one or more heteroatoms, Preferable R^{24} may be cyclo(C_{5-7})alkyl group optionally having suitable substituents, and the following ones can be exemplified.

(a) a 3,4-di-oxo-cyclohexyl group;

(b) a 3-R^{20}-4-R^{21}-cyclohexyl group,

in which R^{20} is hydroxy, an alkoxy group, an oxo group, or a $-OCH_2OCH_2CH_2OCH_3$ group, and

R^{21} is hydroxy, -OCN, an alkoxy group, a heteroaryloxy which may be substituted by suitable substituents, 1- or 2-tetrazolyl, a $-OCH_2OCH_2CH_2OCH_3$ group, a protected hydroxy group, chloro, bromo, iodo, aminooxalyloxy, an azido group, p-tolyloxythiocarbonyloxy, or $R^{25}R^{26}CHCOO-$,

in which R^{25} is optionally protected hydroxy or protected amino, and

R^{26} is hydrogen or methyl, or

R^{20} and R^{21} together form an oxygen atom in an epoxide ring; or

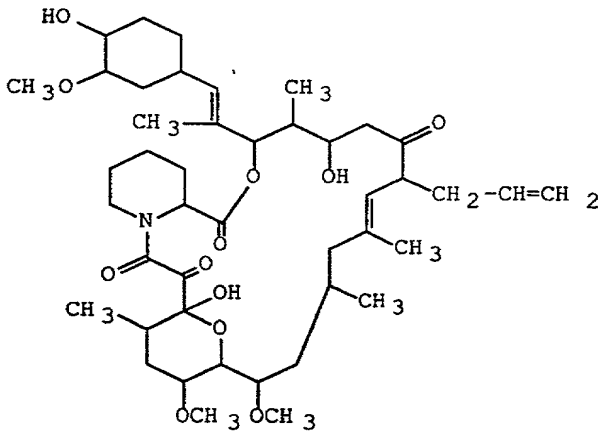
(c) cyclopentyl group substituted by methoxymethyl, optionally protected hydroxymethyl, acyloxymethyl (in which the acyl moiety optionally contains either a dimethylamino group which may be quaternized, or a carboxy group which may be esterified), one or more amino and/or hydroxy groups which may be protected, or aminooxalyloxymethyl. A preferred example is a 2-formyl-cyclopentyl group.

"A heteroaryl which may be substituted by suitable substituents" moiety of the "heteroaryloxy which may be substituted by suitable substituents" may be the ones exemplified for R^1 of the compound of the formula of EP-A-532,088, with preference given to 1-hydroxyethylindol -5-yl, the disclosure of which is incorporated herein by reference.

The ticyclic compounds (I) and its pharmaceutically

acceptable salt for use in accordance with this invention are well known to have excellent immunosuppressive activity, antimicrobial activity and other pharmacological activities and, as such, be of value for the treatment or prevention of rejection reactions by transplantation of organs or tissues, graft-vs-host diseases, autoimmune diseases, and infectious diseases [EP-A-0184162, EP-A-0323042, EP-A-423714, EP-A-427680, EP-A-465426, EP-A-480623, EP-A-532088, EP-A-532089, EP-A-569337, EP-A-626385, WO89/05303, WO93/05058, WO96/31514, WO91/13889, WO91/19495, WO93/04680, WO93/5059, etc.], the disclosures of which are incorporated herein by reference.

Particularly, the compounds which are designated as FR900506 (=FK506), FR900520 (ascomycin), FR900523, and FR900525 are products produced by microorganisms of the genus Streptomyces, such as Streptomyces tsukubaensis No. 9993 [deposited with National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology (formerly Fermentation Research Institute Agency of Industrial Science and Technology), at 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, date of deposit October 5, 1984, accession number FERM BP-927] or Streptomyces hygroscopicus subsp. yakushimaensis No. 7238 [deposited with National Institute of Bioscience and Human Technology Agency of Industrial Science and Technology (formerly Fermentation Research Institute Agency of Industrial Science and Technology), at 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki, Japan, date of deposit January 12, 1985, accession number FERM BP-928][EP-A-0184162]. The FK506 (general name: tacrolimus) of the following chemical formula, in particular, is a representative compound.



Chemical name: 17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylvinyl]-23,25-dimethoxy-13,19,21,27-tetramethyl-11,28-dioxa-4-azatricyclo[22.3.1.$0^{4,9}$]octacos-18-ene-2,3,10,16-tetraone

The preferred examples of the tricyclic compounds (I) are the ones, wherein each of adjacent pairs of R^3 and R^4 or R^5 and R^6 independently form another bond formed between the carbon atoms to which they are attached;

each of R^8 and R^{23} is independently a hydrogen atom;

R^9 is a hydroxy group;

R^{10} is a methyl group, an ethyl group, a propyl group or an allyl group;

X is (a hydrogen atom and a hydrogen atom) or an oxo group;

Y is an oxo group;

each of R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19}, and R^{22} is a methyl group;

R^{24} is a 3-R^{20}-4-R^{21}-cyclohexyl group,

in which R^{20} is hydroxy, an alkoxy group, an oxo group, or a $-OCH_2OCH_2CH_2OCH_3$ group, and

R^{21} is hydroxy, -OCN, an alkoxy group, a heteroaryloxy which may be substituted by suitable substituents, 1- or 2-tetrazolyl, a $-OCH_2OCH_2CH_2OCH_3$ group, a protected hydroxy group, chloro, bromo, iodo, aminooxalyloxy, an azido group, p-tolyloxythiocarbonyloxy, or $R^{25}R^{26}CHCOO-$,

in which R^{25} is optionally protected hydroxy or protected amino, and

R^{26} is hydrogen or methyl, or

R^{20} and R^{21} together form an oxygen atom in an epoxide ring; and

n is an integer of 1 or 2.

The most preferable tricyclic compounds(I) is, in addition to FK506, ascomycin derivatives such as halogenated-ascomycin (e.g., 33-epi-chloro-33-desoxyascomycin), which is disclosed in EP 427,680, example 66a.

As the other preferable example of the IL-2 inhibitor, rapamycin [THE MERCK INDEX (12th edition), No. 8288] and its derivatives can be exemplified. Preferred example of the derivatives is an O-substituted derivative in which the hydroxy in position 40 of formula A illustrated at page 1 of WO 95/16691, incorporated herein by reference, is replaced by $-OR_1$ in which R_1 is hydroxyalkyl, hydroalkoxyalkyl, acylaminoalkyl and aminoalkyl; for example 40-O-(2-hydroxy)ethyl-rapamycin, 40-O-(3-hydroxy)propyl-rapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethyl-rapamycin and 40-O-(2-acetaminoethyl)-

rapamycin. These O-substituted derivatives may be produced by reacting rapamycin (or dihydro or deoxo-rapamycin) with an organic radical attached to a leaving group (for example RX where R is the organic radical which is desired as the O-substituent, such as an alkyl, allyl, or benzyl moiety, and X is a leaving group such as $CCl_3C(NH)O$ or CF_3SO_3) under suitable reaction conditions. The conditions may be acidic or neutral conditions, for example in the presence of an acid like trifluoromethanesulfonic acid, camphorsulfonic acid, p-toluenesulfonic acid or their respective pyridinium or substituted pyridinium salts when X is $CCl_3C(NH)O$ or in the presence of a base like pyridine, a substituted pyridine, diisopropylethylamine or pentamethylpiperidine when X is CF_3SO_3. The most preferable one is 40-O-(2-hydroxy)ethyl rapamycin, which is disclosed in WO94/09010, the disclosure of which is incorporated herein by reference.

The tricyclic compounds(I), and rapamycin and its derivatives, may be in a form of its salt, which includes conventional non-toxic and pharmaceutically acceptable salt such as the salt with inorganic or organic bases, specifically, an alkali metal salt such as sodium salt and potassium salt, an alkali earth metal salt such as calcium salt and magnesium salt, an ammonium salt and an amine salt such as triethylamine salt and N-benzyl-N-methylamine salt.

With respect to the IL-2 inhibitor of the present invention, particularly the tricyclic macrolide compounds, it is to be understood that there may be conformers and one or more stereoisomers such as optical and geometrical isomers due to

asymmetric carbon atom(s) or double bond(s), and such conformers and isomers are also included within the scope of the present invention. And further, the tricyclic macrolide compounds can be in the form of a solvate, which is included within the scope of the present invention. The solvate preferably include a hydrate and an ethanolate.

Further example of the IL-2 inhibitor is cyclosporin and its derivatives such as cyclosporin A, B, C, D, E, F, G, etc, which are shown in THE MERCK INDEX (12th edition), No. 2821, USP 4,117,118, 4,215,199, 4,288,431, 4,388,307, Helv. Chim. Acta. 60, 1568(1977) and 65, 1655(1982), Transplant. Proc. 17, 1362(1985), and so on. Among which, the most preferable one is cyclosporin A. The disclosures of the above references are incorporated herein.

The tricyclic compounds (I) and its pharmaceutically acceptable salts, and cyclosporin or its derivatives may be classified as "IL-2 production inhibitor", which show immunosuppressive activity by inhibiting the production of IL-2. And rapamycin or its derivatives may be classified as " IL-2 signal transmission inhibitor", which show immunosuppressive activity by inhibiting the transmission of IL-2 signal.

For therapeutic administration, plasminogen activators in the present invention is used in the form of a conventional pharmaceutical preparation in admixture with a conventional pharmaceutically acceptable carrier such as an organic or inorganic solid or liquid excipient which is suitable for oral, parenteral or external administration. The pharmaceutical

preparation may be compounded in a solid form such as granule, capsule, tablet, dragee, suppository or ointment, or in a liquid form such as solution, suspension or emulsion for injection, intravenous drip, ingestion, eye drop, etc. If needed, there may be included in the above preparation auxiliary substance such as stabilizing agent, wetting or emulsifying agent, buffer or any other commonly used additives.

The plasminogen activators as the effective ingredient may usually be administered in an amount which can activate plasminogen to plasmine. In particular, it may be a unit dose of 0.001 mg/kg to 500 mg/kg, preferably 0.01 mg/kg to 10 mg/kg,1 to 4 times a day. However, the above dosage may be increased or decreased according to age, body weight and conditions of the patient or administering method.

For applying this composition to a human, it is preferable to apply it by injection.

In the present invention, a plasminogen activator is able to be administered for increasing the effect caused by a IL-2 inhibitor simultaneously, separately or in sequential use with a IL-2 inhibitor.

If advisable, the plasminogen activators can be mixed with the IL-2 inhibitor prior to its use. So, the composition comprising the said plasminogen activators of the present invention may further comprise the IL-2 inhibitor. And optionally, it comprises further additional ingredients, such as, mycophenolate mofetil (CellCept), steroids, Azathiopurine, and so on.

While the effective dosage of the IL-2 inhibitor depends on the type of the said IL-2 inhibitor, the patient's age, type

of disease, severity of illness, and other factors, a daily dose thereof is about 0.01~1000 mg, preferably 0.05~500 mg, and more preferably, 0.1~100 mg for therapeutic purposes. The average unit dose may be generally about 0.1 mg, 0.5 mg, 1 mg, 5 mg, 10 mg, 50 mg, 100 mg, 250 mg, or 500 mg.

The following Preparations and Examples are given for the purpose of illustrating the present invention in detail.

Example 1

Effect of the combination therapy of FK506 and a tissue-type plasminogen activator in a rat middle cerebral artery thrombosis model:

Method)

Animal preparation

Thrombotic occlusion of the MCA was induced by photochemical reaction as described by Umemura et al., (1993). Briefly, male Sprague-Dawley rats (SLC, Inc.) weighting about 300g were anesthetized with halothane (4% for induction, 1.5% for maintenance). The animals were placed in the lateral position, and the left MCA was exposed by a microsurgical approach. A stable thrombotic occlusion of MCA was produced by photochemical reaction between intravenously administered photoreactive dye, rose bengal (10 mg/kg) and transmural green light(540nm), which causes endothelial injury followed by platelet activation. The body temperature of animals was maintained at 37.0~38.0 °C using a heating-pad. Twenty-four hours after the ischemic insult, the brain was removed for histopathological assessment with

triphenyltetrazolium chloride(TTC) staining. The infarct area was calculated by a computerized image analysis system.

Drug treatment

FK506(1 mg/kg; Prograf(Trade Name:Fujisawa pharmaceutical Co., Ltd.) was administered intravenously by a single bolus injection through the femoral vein 2 hours after occlusion of the MCA. A tissue-type plasminogen activator (t-PA)(1 mg/kg), which is a recombinant t-PA, 'alteplase', was administered intravenously by a bolus injection (20 % of total volume) followed by infusion (80 % of total volume) for 30 min through the femoral vein 2 hours after occlusion of the MCA. In the combination study, following the administration of FK506, t-PA (1 mg/kg) was administered as described above.

Results)

Therapeutic efficacy of the combination of FK506 and t-PA:

When drugs were administered 2 hours after occlusion of the MCA, FK506 or t-PA showed a relatively small tendency of the inhibition of brain damage. However, the combination of FK506 and t-PA caused the significant reduction of ischemic brain damage and its inhibition is more than 23%, which is greater than that of FK506 or t-PA alone.

Additionally, when drugs were administered 3 hours after occlusion of the MCA, t-PA increased the brain damage(-13.8± 7.0%). On the contrary, the combination of FK506 and t-PA caused the significant reduction of ischemic brain damage and its inhibition is 16.2±7.6%.

These results indicate that FK506 is able to decrease the

serious damages caused by t-PA. In other word, the above results indicate that the combination of FK506 and t-PA not only prolongs the therapeutic time window but also produces increased efficacy and safety for treatment of the ischemic brain damage.

So, the present invention provides useful neuroprotective agent for preventing or treating acute or chronic cerebral neurodegenerative diseases, such as cerebral ischemic diseases and/or brain damage caused by ischemia. So, it is useful when the following diseases or injury occur, that is, cerebral infarction, head injury, hemorrhage in brain such as subarachnoid hemorrhage or intracerebral hemorrhage, cerebral thrombosis, cerebral embolism, cardiac arrest, stroke (such as acute stroke), transient ischemic attacks (TIA), hypertensive encephalopathy, Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and so on.

From the another aspect, the present invention also provides the following inventions.

i) A use of IL-2 inhibitor for manufacturing a medicament for increasing or decreasing an effect caused by plasminogen activator, in which the effect caused by plasminogen activator is a neuroprotective activity or a brain damage appeared in case that plasminogen activator is administered after its proper therapeutic time.

ii) A use of a IL-2 inhibitor and plasminogen activator for manufacturing a medicament for simultaneous, separate or sequential use for neuroprotective activity.

iii) A method for increasing an effect caused by a

plasminogen activator, by administering an effective amount of IL-2 inhibitor to a human being or an animal.

iv) A method for preventing and treating acute or chronic cerebral neurodegenerative diseases, by administering an effective amount of a plasminogen activator and an effective amount of IL-2 inhibitor to a human being or an animal.

v) A composition comprising a plasminogen activator and IL-2 inhibitor as a combined preparation for simultaneous, separate or sequential use for neuroprotective activity.

vi) An article of manufacture, comprising packaging material and IL-2 inhibitor contained within said packaging material, wherein said IL-2 inhibitor is therapeutically effective for increasing or decreasing an effect caused by plasminogen activator, and
wherein said packaging material comprises a label or a written material which indicates that said IL-2 inhibitor can be used for increasing or decreasing an effect caused by plasminogen activator.

vii) An article of manufacture, comprising packaging material and a plasminogen activator contained within said packaging material, wherein said plasminogen activator is therapeutically effective for increasing an effect caused by IL-2 inhibitor, and wherein said packaging material comprises a label or a written material which indicates that said plasminogen activator can be used for increasing an effect caused by IL-2 inhibitor.

The patents, patent applications and publications cited herein are incorporated by reference.

CLAIMS

1. A use of a plasminogen activator for manufacturing a medicament for increasing an effect caused by IL-2 inhibitor.

2. The use of the claim 1, in which the IL-2 inhibitor inhibits the production of IL-2.

3. The use of the claim 1, in which the IL-2 inhibitor inhibits the activity of IL-2.

4. The use of the claim 1, in which the effect caused by IL-2 inhibitor is a neuroprotective activity.

5. The use of the claim 1, in which the IL-2 inhibitor is tacrolimus or its hydrate, or cyclosporins.

6. A use of a plasminogen activator and IL-2 inhibitor for manufacturing a medicament for simultaneous, separate or sequential use for neuroprotective activity.

7. A method for increasing an effect caused by IL-2 inhibitor, by administering a effective amount of a plasminogen activator to a human being or an animal.

8. A method for preventing and treating acute or chronic cerebral neurodegenerative diseases, by administering a effective amount of a plasminogen activator and an effective amount of IL-2 inhibitor to a human being or an animal.

9. A composition comprising a plasminogen activator, for increasing an effect caused by IL-2 inhibitor.

10. A composition comprising a plasminogen activator and IL-2 inhibitor as a combined preparation for simultaneous, separate or sequential use for neuroprotective activity.

11. An article of manufacture, comprising packaging material and a plasminogen activator contained within said packaging material,

wherein said plasminogen activator is therapeutically effective for increasing an effect caused by IL-2 inhibitor, and
wherein said packaging material comprises a label or a written material which indicates that said plasminogen activator can be used for increasing an effect caused by IL-2 inhibitor.

12. A use of IL-2 inhibitor for manufacturing a medicament for increasing or decreasing an effect caused by plasminogen activator, in which the effect caused by plasminogen activator is a neuroprotective activity or a brain damage appeared in case that plasminogen activator is administered after its proper therapeutic time.

13. A method for increasing or decreasing an effect caused by plasminogen activator, by administering a effective amount of IL-2 inhibitor, in which the effect caused by plasminogen activator is a neuroprotective activity or a brain damage appeared in case that plasminogen activator is administered after its proper therapeutic time.

14. A composition comprising IL-2 inhibitor, for increasing or decreasing an effect caused by plasminogen activator.

15. An article of manufacture, comprising packaging material and IL-2 inhibitor contained within said packaging material, wherein said IL-2 inhibitor is therapeutically effective for increasing or decreasing an effect caused by plasminogen activator, and
wherein said packaging material comprises a label or a written material which indicates that said IL-2 inhibitor can be used for increasing or decreasing an effect caused by plasminogen activator.

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(54) Title: NEW USE

(57) Abstract: The present invention is related to a new use of a plasminogen activator, for increasing an effect caused by IL-2 inhibitor and, further, a use of IL-2 inhibitor for increasing or decreasing an effect caused by plasminogen activator.

Declaration, Power of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NEW USE

the specification of which

☐ is attached hereto.

☐ was filed on _____ as

Application Serial No. _____

and amended on _____.

☒ was filed as PCT international application

Number PCT/JP00/06874

on October 2, 2000,

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
PQ3249	Australia	04/10/99	☒ Yes ☐ No
PQ5643	Australia	15/02/00	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	**Filing Date**	**Status (pending, patented, abandoned)**
PCT/JP00/06874	October 2, 2000	

And we (I) hereby appoint the following registered practitioner(s):

22850

as our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to

22850

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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